

SHORT REPORT

Correlation between culture testing of swabs and ligase chain reaction of first void urine from patients recently treated for *Chlamydia trachomatis*

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We assessed the correlation between ligase chain reaction (LCR) on first void urine (FVU) and cultures of urethral and cervical swabs to detect chlamydia during three post-treatment follow up visits for 10 men and 19 women with genital chlamydial infections who had been treated with azithromycin or doxycycline.

The most common sexually transmitted bacterial infection in North America is *Chlamydia trachomatis*.¹ Highest rates of infections occur among young adults aged 15–24, and rates are often higher in females than males. Infections are often asymptomatic, and if untreated, may lead to upper genital tract complications of proctitis and epididymitis in men, and pelvic inflammatory disease, endometritis, salpingitis, ectopic pregnancy, and tubal factor infertility in women.² Rates of recurrence or persistence of chlamydial infection in young women have been estimated to range from 4.1% to 13.4%.³

Current treatment guidelines in Canada and the United States for *C trachomatis* infections recommend oral treatment with a 7 day course of doxycycline or a single dose of azithromycin. Studies have demonstrated comparable cure rates for both single and multidose therapy.⁴ Ligase chain reaction (LCR) testing of first void urine (FVU) to detect *C trachomatis* is an alternative to swab testing, especially in settings where most individuals are asymptomatic, or where patients are unlikely to agree to a pelvic examination or urethral swab.⁵ Other nucleic acid amplification (NAA) tests such as polymerase chain reaction (PCR) and transcription mediated amplification (TMA) have demonstrated similar efficacy on FVU and some data are available on the role of NAA assays as tests of cure.^{6–8}

METHODS

Patient selection

Ten men and 19 women, who were confirmed positive for *C trachomatis* by EIA or culture and were positive in FVU tested by LCR at baseline, were recruited from STD clinics which participated in a multicentre randomised controlled trial in Vancouver, British Columbia, Sherbrooke, Quebec, Laval, Quebec, and Regina, Saskatchewan. That trial compared the efficacy, safety, and tolerance of azithromycin (1 g single dose) and doxycycline (100 mg twice daily for 7 days). Men with symptoms (urethral discharge, dysuria or urethral irritation) and a urethral smear negative for *Neisseria gonorrhoeae*, showing ≥ 4 polymorphonuclear neutrophils (PMN) per high power field were eligible. Women with an endocervical smear showing ≥ 10 PMN per high power field or with a positive chlamydia enzyme immunoassay (EIA) or culture were also

eligible. All subjects gave written informed consent, which was approved by the institutional review board at each participating institution.

All 29 patients returned for at least two of the three follow up visits at 8, 28, and 42 days after treatment had begun.

Clinical visits

At the initial visit each patient underwent a physical examination and a medical history was taken. A clinical examination was performed, documenting urethral discharge, dysuria, and urethral irritation in men and purulent or mucopurulent discharge, cervical erythema, oedema, friability, or hypertrophic ectopy in women. Swabs for *C trachomatis* and *N gonorrhoeae* testing by culture and EIA were collected from the endocervix of the women and from the urethra of the men.

Patients were followed up at the end of three successive periods at 8, 28, and 42 days after the initiation of antibiotic treatment to determine chlamydia status. Urethral and cervical swabs were obtained from men and women respectively for chlamydia culture and FVU (the first 25 ml of urine when voiding) was obtained for LCR testing.

Laboratory procedures

At study admission, urethral and cervical specimens were tested by culture or EIA (Chlamydiazyme, Abbott Laboratories, Chicago, IL, USA) at each of the trial sites. For culture, specimens were shipped to the laboratories on wet ice and inoculated onto McCoy cells either in microtitre plates or shell vials and stained for inclusions using fluorescent labelled species specific monoclonal antibody for *C trachomatis* cultures⁹ and selective chocolate agar followed by biochemical testing for identification of *N gonorrhoeae*.⁹ Swab specimens were tested by EIA following the manufacturer's instructions and positives required a confirmatory blocking procedure supplied by Abbott Laboratories. FVU specimens were collected in sterile containers and frozen at -20°C at each site until shipped to the regional virology and chlamydiology laboratory in Hamilton for testing by LCR as described previously.⁵

Genotyping by restriction fragment length polymorphism (RFLP) of the outer membrane protein gene was performed at the microbiology laboratory, University of Sherbrooke, Quebec, on the specimens from first and subsequent visits of participants positive at any of the follow up visits, using previously described methods.¹⁰

RESULTS

The mean age of the 29 patients enrolled with chlamydial infection was 25.2 years (SD 7.1). Almost one third (27.6%; 8/29) reported a previous *C trachomatis* infection and 6.9% (2/29) reported a previous *N gonorrhoeae* infection. The median number of lifetime sexual partners reported was 10. Approximately one third (27.6%; 8/29) reported never using condoms.

Table 1 Patterns of positivity for *C trachomatis* on follow up for three men treated with azithromycin

ID	Assay results	Original	Follow up visits at:		
			8 days	28 days	42 days
64	LCR	+(K)*	–	+(K)	+(K)
	Culture	+	–	–	+
72	LCR	+(E)*	–	+(E)	Lost to follow up
	Culture	+	–	+	
78	LCR	+(NA)*	+(NA)	–	–
	Culture	+	–	–	–

*Restriction fragment length polymorphism PCR of OMP gene shown to be serovar E or K; NA sample did not amplify in RFLP PCR.

All of the patients who received doxycycline treatment (four men and eight women) and all of the 11 women taking azithromycin were free of infection by urethral and cervical culture and FVU LCR at all follow up visits (8, 28, and 42 days after initial visit). Among the men treated with azithromycin (n=6) three were free of infection by culture and FVU LCR at all follow up visits (table 1). One man (patient 78) was FVU positive by LCR but negative by urethral culture at the first follow up visit and negative for both tests in the remaining visits. Patient number 72 was negative by both tests at the first follow up visit then became LCR and culture positive at the second follow up visit but was lost to follow up for the third visit. At the initial follow up visit, patient number 64 was negative by both culture and LCR. At the second follow up visit this patient had a positive FVU by LCR but a negative urethral culture, which preceded positive findings in both samples on the final visit.

To determine if persistence or possible re-infection occurred in the azithromycin treated men who were positive at follow up visits, samples from the original and follow up visits were genotyped by RFLP. The three samples from patient 64 were typed as serovar K. The two samples from patient 72 were type E, and both the original and first follow up visit samples from patient 78 did not amplify to allow typing.

DISCUSSION

As shown by LCR FVU and swab cultures, clearance of *C trachomatis* occurred by 8 days post-treatment for all of the patients receiving doxycycline treatment and most of the patients treated with azithromycin. Clearance of DNA appeared to take longer in the one man taking azithromycin (No 78 in table 1), in whom LCR FVU was positive but culture of the urethral swab was negative at the first follow up visit. This may have been a false negative culture due to suboptimal processing of a swab sample containing very little infectious material. Alternatively, the sample contained no infectious organisms but contained small amounts of persisting DNA. Other studies have shown DNA persistence by PCR and LCR from 1–2 weeks, when viable organisms are difficult to retrieve.^{6,7} This assumption is corroborated by the inability to amplify DNA by PCR in the RFLP studies performed on the samples of patient 78. We were unable to perform LCR on the negative tissue culture remnants to detect *C trachomatis* plasmid DNA from non-viable bacteria. In two other men taking azithromycin (patients 64 and 72), it appeared that the infection was cleared at the first follow up visit, but reappeared at the second and third visits. These men were either re-infected with the same serovar, or had persistent infections without DNA or viable organisms at the first follow up visit. This could be accounted for by amplification inhibitors¹¹ and low levels of viable organisms, which might be inactivated during specimen transit. In a study examining *C*

trachomatis nucleic acids in cervical swabs and urine, Morr   *et al*⁸ demonstrated that DNA measured by PCR persisted in cervical swabs for up to 3 weeks after treatment with doxycycline or azithromycin but RNA measured by nucleic acid sequence based amplification (NASBA) was absent between 1 and 2 weeks post-treatment in both specimens. They observed that DNA did not persist in urine beyond the 7–14 day interval, suggesting to them that this makes urine a potentially suitable specimen for test of cure. Similar to our observations, the study by Morr   *et al* also recorded that following 2 weeks without *C trachomatis* DNA reappeared in newly acquired specimens after 1–2 weeks without DNA.⁸ Consistent with previous findings that NAA assays are generally more sensitive than culture,¹² we found that in patient 64 LCR FVU detected the infection before it was subsequently detected by culture at the next follow up visit. Because of the generally high rates of cure following currently recommended therapies, test of cure is not recommended following treatment of uncomplicated *C trachomatis* infections. However, the observations from this study suggest that when test of cure is considered (complicated cases or recurrent symptoms) LCR performed on FVU may be a convenient and accurate way to monitor antibiotic success or failure in eradicating *C trachomatis* from the lower genital tract. Larger prospective, post-treatment studies are required to substantiate this premise.

CONTRIBUTORS

DJ, JS, MH, JM, and MC designed the study, analysed the data and contributed to writing the paper; JS, DP, CB, JD, and LS provided patient samples; EF performed the CT genotyping.

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